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(54) Title: BIMODAL EMULSIONS OF TAXANES AND BUTYRATE POLYALCOHOL ESTERS

(57) Abstract: The invention provides bimodal emulsions of a taxane and a butyrate polyalcohol ester, and methods for using such emulsions, for the treatment of hyperproliferative disease such as cancer. The emulsions are stabilized by the presence of an Ostwald ripening stabilizer, which reduces Ostwald ripening of the emulsions.

BIMODAL EMULSIONS OF TAXANES AND BUTYRATE POLYALCOHOL ESTERS

BACKGROUND OF THE INVENTION

Paclitaxel was first isolated in 1971 from the bark of the Pacific Yew tree (*Taxus brevifolia*). Subsequently, paclitaxel was approved for treatment of metastatic ovarian cancer and later for breast cancer. The mechanism of action of paclitaxel is believed to involve promoting formation and hyperstabilization of microtubules, thereby preventing the disassembly of microtubules necessary for completion of cell division. Thus, paclitaxel can be classified as an anti-mitotic or anti-proliferative agent. Paclitaxel has also been reported to induce expression of cytokines, to affect the activity of kinases, and to block processes essential for metastasis, in as yet uncharacterized mechanisms of action.

Paclitaxel has attracted the interest of the medical community. This interest is due not only to the unusual antimitotic properties of paclitaxel, but also because it is active against nearly all cancers against which it has been tested. Paclitaxel is a member of a class of related compounds, the taxanes. These compounds are now recognized as a potent class of anticancer compounds.

The use of taxanes to treat hyperproliferative diseases is greatly limited by their low water solubility. Taxanes are difficult to administer intravenously in therapeutically effective dosages. Due to the low solubility of taxanes in aqueous solutions, intravenous therapies using taxanes require extremely long delivery periods (e.g., 3-12 hours) and high volumes of fluid (e.g., a liter or more of IV solution). Such long time periods and low concentration delivery are disadvantageous due to the instability of taxanes in aqueous solutions. Solubilizers, such as Cremophor (polyethoxylated castor oil) and alcohol, improve the solubility but lead to serious side effects, including shock.

Taxanes have been formulated in liposomes to improve their solubility. For example, paclitaxel can be dissolved in the lipid phase of liposomes. (See, e.g., Lundberg, J. Pharm. Pharmacol. 49:16-21 (1997).) While liposomes effectively solubilize small amounts of taxanes, the limited ability of liposomes to solubilize larger

amounts of taxanes is a significant drawback of such methods. (See, e.g., Lundberg, supra; Simamora et al., PDA J. Pharm. Sci. Tech. 52:170-72 (1998).)

Taxanes have also been administered as "water in oil" emulsions. Such emulsions typically comprise an aqueous (continuous) phase and an oil (dispersed) phase. The oil (dispersed) phase typically comprises "droplets" or "particles" of oil-containing paclitaxel. For example, emulsions can be formed with paclitaxel and medium chain and/or long chain fatty acids. (See, e.g., Kan et al., J. Controlled Release 58:271-78 (1999); U.S. Patent No. 5,616,330; the disclosures of which are incorporated by reference herein.) Medium and long chain length triglycerides have poor abilities to solubilize paclitaxel, however. (See, e.g., Kan et al., supra.) Shorter chain length triglycerides reportedly have greater abilities to solubilize paclitaxel in an emulsion. However, emulsions of paclitaxel and the short chain length triglyceride fatty acid triacetin (glycerol triacetate) are unstable, and precipitation of paclitaxel from aqueous emulsions has been reported. (See, e.g., Tarr et al., Pharm. Res. 4:162-65 (1987); Lundberg, J. Pharmacy Pharmacol. 49:16-21 (1997).) Various attempts to control emulsion stability have been unsuccessful. For example, paclitaxel emulsions containing triacetin, L-α-lecithin, polysorbate 80, Pluronics F-68, ethyl oleate and glycerol have been formulated. While such emulsions are reportedly more stable, they are highly toxic. (See Tarr et al., supra.)

Another short chain length triglyceride, tributyrin, is of interest for the treatment of hyperproliferative disease because its substituent, butyrate, is believed to be a cytodifferentiation agent capable of inhibiting the growth of neoplastic cells and inducing apoptosis of such cells. (See, e.g., Chen and Breitman, Cancer Res. 54:3494-99 (1994); Heerdt et al., Cancer Res. 59:1584-91 (1999); U.S. Patent No. 5,645,852).) Butyrate has a short half life in vivo and is typically active in millimolar concentrations, that may be difficult to achieve in a subject. (See, e.g., U.S. Patent No. 5,645,852; Siavoshian et al., Gut 46:507-14 (2000). To achieve higher dosages of butyrate, the compound can be administered as a glycerol ester, tributyrin. (See, e.g., Chen and Breitman, supra; Heerdt et al., supra; U.S. Patent No. 5,645,852.) Like other short chain triglycerides, tributyrin is a better solubilizing agent for paclitaxel. Tributyrin-paclitaxel emulsions are unstable, however, paclitaxel precipitates from the emulsion. (See, e.g., Kan et al., supra; Lundberg, J. Pharmacy Pharmacol. 49:16-21 (1997).) In an effort to improve the performance of paclitaxel-tributyrin emulsions, paclitaxel and tributyrin have been formulated with polysorbate 80, and sorbitan monolaurate. (See, e.g., Simamora et

al., PDA J. Pharm. Sci. & Tech. 52:170-72 (1998). These modified emulsions are also unstable about 3-5 hours after dilution. (See Simamora et al., supra.)

Emulsions of paclitaxel and triglycerides (e.g., tributyrin) can become unstable for a variety of reasons. Factors causing instability of such emulsions include: (1) external forces (e.g., gravitational or centrifugal); (2) flocculation resulting from attractive forces between oil "droplets" in the emulsion; (3) mechanical aggregation of droplets to form larger droplets and ultimately immiscible bulk liquids; (4) "Ostwald ripening" of the emulsion, by which a component of the dispersed phase (e.g., a tributyrin) migrates through the continuous phase between particles of the non-aqueous (dispersed) phase, thus causing the largest droplets or particles to increase in size at the expense of the smallest droplets or particles, which decrease in size; and (5) coalescence resulting from a combination of these factors.

Of these forces, Ostwald ripening may be a significant factor in determining the efficacy of taxane emulsions. As discussed above, Ostwald ripening occurs when a component of the dispersed phase is transferred through the continuous phase from one droplet or particle to another. The usual mechanism for such transfer is by dissolution of the component into the continuous phase from one droplet or particle, followed by fusion of the dissolved component with another droplet or particle. Such transfer can occur even if the solubility of the component is low. Other transfer mechanisms are possible, however. For example, even components that have a very low water solubility, which might not be expected to display Ostwald ripening, can do so when certain surfactants are used in the preparation and stabilization of the emulsion.

The direction of migration of the non-aqueous phase tends to be from smaller particles to larger particles, because of the respective chemical potential of the non-aqueous components in the emulsion particles. Thus, the overall effect of the migration of components between the emulsion particles is to tend to cause the particle size distribution to shift towards larger particle sizes, which is disadvantageous in many cases. Such migration can lead to coalescence and other forms of emulsion instability.

Thus, there is an urgent need in the art for stable taxane-butyrate emulsions that are capable of delivering therapeutic amounts of taxanes and butyrates to a subject to treat hyperproliferative disease. The present invention surprisingly satisfies these needs and more.

SUMMARY OF THE INVENTION

The invention provides stabilized bimodal emulsions comprising a taxane and a butyrate polyalcohol ester. The taxane and butyrate polyalcohol ester are effective against a wide variety of hyperproliferative diseases, such as breast cancer and metastatic ovarian cancer. In more detailed aspects, the invention further comprises a stabilizer, for example, an oil, typically a medium chain fatty acid, to reduce Ostwald ripening of the emulsion.

Accordingly, in a first aspect of the invention, there is provided a bimodal emulsion comprising an aqueous (continuous) phase and a non-aqueous (dispersed) phase. The non-aqueous phase comprises a taxane and a butyrate polyalcohol ester. The butyrate polyalcohol ester solubilizes the taxane in the non-aqueous phase. The butyrate polyalcohol ester is capable of transfer through the aqueous phase between particles of the non-aqueous phase to cause Ostwald ripening of the emulsion. The transfer of the butyrate polyalcohol ester through the aqueous phase is reduced by an Ostwald ripening stabilizer, which is present in an amount effective to reduce transfer of the butyrate polyalcohol ester through the aqueous phase. The aqueous phase can be selected from water, a saline solution, a dextrose solution, a glycerol solution, and the like. In a preferred embodiment, the butyrate polyalcohol ester is present in an amount effective to induce apoptosis and/or cytodifferentiatation of cells in the subject. The emulsion optionally further comprises a surfactant, which reduces the surface tension between the non-aqueous phase and the aqueous phase.

In another aspect, a non-aqueous phase is provided which comprises a mixture of a taxane, a butyrate polyalcohol ester and an Ostwald ripening stabilizer. The butyrate polyalcohol ester solubilizes the taxane in the non-aqueous mixture. The Ostwald ripening stabilizer reduces the transfer of the butyrate polyalcohol ester when the mixture is added to an aqueous solution to form a bimodal emulsion according to the present invention.

The taxane comprises at least one taxane, derivative or analog thereof, which acts as an anti-mitotic agent. The taxane or taxane derivative or analog can include for example, paclitaxel (TAXOL®); taxotere; spicatin; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone, acetate; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-,cyclic 9, 10-acetal with acetone; taxane-2β, 5β, 9β, 10β-tetrol, cyclic 9, 10-

acetal with acetone; taxane; cephalomannine-7-xyloside; 7-epi-10-deacetylcephalomannine; 10-deacetylcephalomannine; cephalomannine; taxol B; 13-(2',3'-dihydroxy-3'-phenylpropionyl)baccatin III; yunnanxol; 7-(4-azidobenzoyl)baccatin III; N-debenzoyltaxol A; O-acetylbaccatin IV; 7-(triethylsilyl)baccatin III; 7, 10-Di-O-[(2,2,2-trichloroethoxy)carbonyl]baccatin III; baccatin III 13-O-acetate; baccatin diacetate; baccatin; baccatin VII; baccatin VI; baccatin IV; 7-epi-baccatin III; baccatin V; baccatin I; baccatin III; baccatin A; 10-deacetyl-7-epitaxol; epitaxol; 10-deacetyltaxol C; 7-xylosyl-10-deacetyltaxol; 10-deacetyltaxol-7-xyloside; 7-epi-10-deacetyltaxol; 10-deacetyltaxol; or 10-deacetyltaxol B, as well as any combination of two or more of the foregoing molecules which are admixed or combined in an emulsion formulation.

The butyrate polyalcohol ester comprises an ester of butyrate and a polyalcohol, such as for example, tributyrin, tetrabutyral butanol, glycerol dibutyrate, tetrabutyral pentanol, or pentabutyral pentanol. The Ostwald ripening stabilizer generally comprises an oil, such as a medium chain length triglyceride, such as a C_{16}-C_{20} triglyceride, or a long chain length triglyceride, such as a C_{22}-C_{24} triglyceride. In exemplary embodiments, the Ostwald ripening stabilizer is triolein (glycerol trioleic acid), tristearin (glycerol tristearic acid), trilinolein (glycerol trilinoleic acid), glycerol trielaidic acid, tripalmitin (glycerol tripalmitic acid), and the like. In other embodiments, the surfactant can be an anionic, cationic, nonionic, zwitterionic or ampholytic surfactant, and is typically a pharmaceutically acceptable surfactant.

In another aspect, methods of preparing bimodal emulsions are provided. Such methods typically comprise preparing an aqueous (continuous) phase, and preparing a non-aqueous (dispersed) phase. The non-aqueous phase is formed by combining a taxane, a butyrate polyalcohol ester and an Ostwald ripening stabilizer. The bimodal emulsion is formed, for example, by dispersing the non-aqueous phase comprising the taxane, butyrate polyalcohol ester and stabilizer. Alternatively, the bimodal emulsion is formed by mixing the surfactant into an aqueous phase and then dispersing the taxane, butyrate polyalcohol ester and Ostwald ripening stabilizer into the aqueous phase such that the butyrate polyalcohol ester, taxane and stabilizer are incorporated in the non-aqueous phase of the emulsion.

In another aspect of the invention, methods for treating hyperproliferative disease are provided. The methods generally comprise administering to a subject in need thereof a therapeutically effective amount of a stable bimodal emulsion, the emulsion comprising a continuous aqueous phase and a dispersed non-aqueous phase, the non-

aqueous phase comprising a taxane, a butyrate polyalcohol ester, and an Ostwald ripening stabilizer. The Ostwald ripening stabilizer, which is soluble in the dispersed phase, but insoluble in the aqueous phase, is present in an amount effective to reduce Ostwald ripening of the emulsion. In a preferred embodiment, the butyrate polyalcohol ester is present in an amount effective to induce apoptosis and/or cytodifferentiation of cells in the subject, and thus act as an anti-mitotic agent.

DEFINITIONS

Prior to setting forth the invention in more detail, it may be helpful to a further understanding thereof to set forth definitions of certain terms used herein.

The term "taxane" refers to an anti-mitotic agent that is a taxane, taxoid, taxine or a derivative or analog thereof.

The term "taxane derivative" refers to a chemically modified taxane, wherein one or more atoms of a taxane are removed or substituted, or new atoms are added. Such modifications include, but are not limited to, removal, substitution or addition of alkyl groups, carbonyl groups, alkyl amino groups, methyl groups, amino groups, amide groups, benzene rings, hydroxyl groups, and the like.

The term "anti-mitotic agent" refers to a compound that inhibits progression of a cell through the cell cycle. Anti-mitotic agents can block progression from, for example, G_1 to S, from S to G_2, from G_2 to M, from M to G_1 or from G_0 to G_1. Anti-mitotic agent can also induce apoptosis.

The term "apoptotic agent" refers to a compound that induces or stimulates apoptotis, which leads to cell suicide, and is characterized by readily observable morphological and biochemical phenomena, such as the fragmentation of the deoxyribonucleic acid (DNA), condensation of the chromatin, margination of cell nuclei, the formation of apoptotic bodies, mitochondrial swelling, and/or dissipation of the mitochondrial proton gradient.

The term "cytodifferentiation agent" refers to a compound that induces differentiation in a hyperproliferative cell, thereby changing the cell phenotype and inhibiting proliferation of the cell.

The term "emulsion" refers to a colloidal dispersion of one immiscible liquid (the dispersed phase) in another liquid phase (the continuous phase). The emulsions of the present invention comprise a continuous aqueous phase (e.g., an aqueous

solution) and a dispersed non-aqueous phase (e.g., at least one immiscible liquid). The non-aqueous phase can comprise droplets or particles of a slightly soluble or insoluble liquids or solids (e.g., a solid solubilized in a non-aqueous liquid in the non-aqueous phase). The term "emulsion" further includes a dispersion, such as a liquid-liquid two phase system in which the non-aqueous (dispersed) phase comprises small particles distributed throughout the aqueous (continuous) phase. The sizes of the droplets or particles in the dispersed phase can range from about 1 to about 4000 nanometers ("nm") or more in diameter, typically from about 50 to about 200 nm in diameter.

The term "biologically active" refers to an amount of a taxane or butyrate polyalcohol ester that effectively modulates the mitotic state of an individual cell, such that mitosis is inhibited, apoptosis is induced or stimulated, differentiation is induced and/or proliferation is stimulated.

The terms "therapeutically useful" or "therapeutically effective" refer to an amount of a taxane or butyrate polyalcohol ester that effectively modulates the state of an individual cell, such that proliferation of one or more cells associated with a hyperproliferative disease is reduced, apoptosis is induced or stimulated, mitosis is inhibited and/or differentiation is induced.

The term "proliferation" refers to activities such as cell division, nuclear division, and other changes in cell state that occur during cellular progression through the cell cycle.

The term "hyperproliferation" refers to an increase in one or more proliferative activities in a cell, as compared with a cell from normal tissue.

The term "hyperproliferative disease" refers to a disease, condition, or disorder associated with hyperproliferation. Diseases involving hyperproliferation include, but are not limited to, cancer (e.g., breast cancer, metastatic ovarian cancer, prostate cancer, and the like), malignancies, premalignant conditions (e.g., hyperplasia, metaplasia, dysplasia), benign tumors, benign dysproliferative disorders, autoimmune diseases, and the like.

The term "transferred" through the aqueous phase means that a compound (e.g., a butyrate polyalcohol ester) is at least partially soluble in the aqueous phase, and that transport of the compound through the aqueous phase from one droplet or particle to another occurs.

The term "not transferred" through the aqueous phase means that the Ostwald ripening stabilizer is essentially insoluble in the aqueous phase, and that transfer

of the stabilizer through the aqueous phase from one droplet or particle to another does not occur to a significant degree. The aqueous solubility of the Ostwald ripening stabilizer in the aqueous phase is less than the solubility of the butyrate polyalcohol ester in that phase and is typically not more than about 100 ppm by weight.

DETAILED DESCRIPTION

This invention provides bimodal emulsions of a taxane and a butyrate polyalcohol ester for the treatment of cancer and other hyperproliferative diseases.

The Bimodal Emulsion

In a first aspect of the invention, there is provided a bimodal emulsion comprising a continuous aqueous phase and a non-aqueous dispersed phase. A butyrate polyalcohol ester solubilizes the taxane in the non-aqueous phase. The butyrate polyalcohol ester is capable of transfer through the aqueous phase to cause Ostwald ripening of the emulsion. In some embodiments, to reduce Ostwald ripening, the non-aqueous phase further comprises an Ostwald ripening stabilizer. The stabilizer is soluble in the non-aqueous phase, but insoluble in and not transferable through the aqueous phase.

The presence of the Ostwald ripening stabilizer in the non-aqueous phase typically has the effect of reducing the net transfer of the butyrate polyalcohol ester through the aqueous phase. Although not intending to be bound by any theory of operation, it is believed that the stabilization of the emulsion is caused by the effect that the Ostwald ripening stabilizer has on the chemical potential of the emulsion. In a normal emulsion (i.e., without a stabilizer present), the butyrate polyalcohol ester in the non-aqueous phase can migrate through the aqueous phase, whether by dissolution in it, or by micelle transfer, and tends to migrate from the smaller to larger size droplets or particles. Migration in this direction is energetically favored because it results in a decrease in the overall free energy of the system. With the stabilizer present, however, this migration is countered, because migration of the butyrate polyalcohol ester from droplets or particles in the non-aqueous phase tends to raise the concentration of the stabilizer in the respective smaller particles, and hence to increase the chemical potential. As the concentration of the stabilizer increases, migration of butyrate polyalcohol ester from the smaller droplets

or particles to larger droplets or particles is energetically disfavored. Thus, the overall effect is that the particle size distribution of the emulsion is stabilized.

The taxane can be any anti-mitotic taxane, taxane derivative or taxane analog. The mechanism of action of taxanes is generally believed to involve promoting formation and hyperstabilization of microtubules, thereby preventing the disassembly of microtubules necessary to complete cell division. Thus, taxanes act late in the cell cycle (between the G_2 and M phases) to block mitosis.

In specific embodiments, the taxane, derivative or analog can include, for example, paclitaxel (TAXOL®); taxotere; spicatin; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone, acetate; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-,cyclic 9, 10-acetal with acetone; taxane-2β, 5β, 9β, 10β-tetrol, cyclic 9, 10-acetal with acetone; taxane; cephalomannine-7-xyloside; 7-epi-10-deacetylcephalomannine; 10-deacetylcephalomannine; cephalomannine; taxol B; 13-(2',3'-dihydroxy-3'-phenylpropionyl)baccatin III; yunnanxol; 7-(4-azidobenzoyl)baccatin III; N-debenzoyltaxol A; O-acetylbaccatin IV; 7-(triethylsilyl)baccatin III; 7, 10-Di-O-[(2,2,2-trichloroethoxy)carbonyl]baccatin III; baccatin III 13-O-acetate; baccatin diacetate; baccatin; baccatin VII; baccatin VI; baccatin IV; 7-epi-baccatin III; baccatin V; baccatin I; baccatin III; baccatin A; 10-deacetyl-7-epitaxol; epitaxol; 10-deacetyltaxol C; 7-xylosyl-10-deacetyltaxol; 10-deacetyltaxol-7-xyloside; 7-epi-10-deacetyltaxol; 10-deacetyltaxol; or 10-deacetyltaxol B, as well as any combination of two or more of the foregoing molecules admixed or combined in a emulsion formulation.

The taxane is solubilized in the non-aqueous phase by a butyrate polyalcohol ester. The butyrate polyalcohol ester is a butyrate polyalcohol ester having at least two butyrate groups esterified to at least two hydroxyl groups of the polyalcohol, the polyalcohol having the general formula $CH_2OH(CHOH)_nCH_2OH$, where n is from 0 to 5, or more. The butyrate polyalcohol ester can also be a mixture of butyrate polyalcohol esters. Suitable butyrate polyalcohol esters include glycerol dibutyrate, glycerol tributyrate (tributyrin), tetrabutyral pentanol, pentabutyral pentanol., and the like. The butyrate polyalcohol ester is typically a pharmaceutically acceptable butyrate polyalcohol ester. In a preferred embodiment, the butyrate polyalcohol ester is a substantially pure butyrate polyalcohol ester. In this context, "substantially pure" means that the butyrate polyalcohol ester is at least about 90 weight percent butyrate polyalcohol ester.

In one embodiment, the butyrate polyalcohol ester is present in an amount sufficient to induce or stimulate apoptosis, and to inhibit mitosis of cells through the cell cycle. Typically, the butyrate polyalcohol ester acts between the G_1 and S phases of the cell cycle by the mechanism of non-reversibly down regulating the expression of the anti-apoptotic Bcl-2 family member proteins (e.g., Bcl-2, Bcl-X_L, Bcl-X_s and Bax). Thus, the action of the butyrate polyalcohol ester complements that of the taxane, each killing cells that escape the action of the other compound. In another embodiment, the butyrate polyalcohol ester is present in an amount sufficient to induce cytodifferentiation of the hyperproliferative cells.

The Ostwald ripening stabilizer is typically a medium chain length triglyceride, such as a C_{16}-C_{20} triglyceride, that is soluble in the non-aqueous phase (i.e., in combination with the taxane and butyrate polyalcohol ester), but insoluble in the aqueous phase. In another embodiment, the stabilizer is a long chain length triglyceride, such as a C_{22}-C_{24} triglyceride. In particular embodiments, the triglyceride is, selected from, for example, triolein (glycerol trioleic acid), tristearin (glycerol tristearic acid), trilinolein (glycerol trilinoleic acid), glycerol trielaidic acid, tripalmitin (glycerol tripalmitic acid). In a preferred embodiment, the stabilizer is substantially pure. In this context, "substantially pure" means that the Ostwald ripening stabilizer is at least about 90 weight percent of a single chain length triglyceride (e.g., 90 weight percent triolein).

In preferred embodiments, the Ostwald ripening stabilizer reduces Ostwald ripening by 5, 10, 15, 20 percent or more, as compared with a composition without the stabilizer. Ostwald ripening is measured by determining the average droplet or particle size (e.g., diameter) in the emulsion, and any change in the droplet size, over the test period. The average particle size can be measured using, for example, an Ostuku LPA-3000 (Otsuka Electronics), a Nicomp 370 or a Horiba CAPA-700 Particle Analyzer. Stability of the emulsion is typically measured in concentrated as well as diluted solutions (e.g., 1 to 10, 1 to 100 and/or 1 to 1000 dilutions). For diluted solutions, the diluents is typically a IV solution, such as can be used for co-administration of the emulsion. The stability can be measured over a period of hours or days for high dilutions, and for periods of weeks or months for concentrated solutions.

The non-aqueous phase can include different ratios of the butyrate polyalcohol ester and the Ostwald ripening stabilizer. The taxane is present in the non-aqueous phase (i.e., the butyrate polyalcohol ester/Ostwald ripening stabilizer mixture) according to the desired dosage of the taxane and the solubility of the taxane in the non-

aqueous phase. In one embodiment, the non-aqueous phase comprises about 0.1 to about 20 mg/ml taxane, more typically about 1 to about 15 mg/ml taxane and preferably about 5 to about 11 mg/ml taxane. In another embodiment, the butyrate polyalcohol ester is present at about 0.5% to about 25% w/v, more typically about 2 to about 15% w/v and preferably about 5 to about 12% w/v. The latter ranges are more preferred to induce apoptosis and/or cytodifferentiation. The ratio of the Ostwald ripening stabilizer to the butyrate polyalcohol ester is typically about 0.001 to about 0.25, more typically about 0.01 to about 0.15, and preferably about 0.04 to about 0.10.

The non-aqueous and/or aqueous phases can optionally include other components, such as preservatives, bioactive agents, and the like, as more fully discussed below. The non-aqueous phase can further comprise a water-immiscible solvent. Such a water-immiscible solvent can be used as a carrier for the taxane, and/or to control the droplet or particle size of the non-aqueous phase when it is dispersed in the aqueous phase. Typical water-immiscible solvents include mineral oils.

In another aspect, the non-aqueous phase is provided with the aqueous (continuous) phase to form a bimodal emulsion according to the present invention. The aqueous (continuous) phase is typically an aqueous solution. Such an aqueous solution can comprise, for example, water, a saline solution (e.g., 0.1%-0.9% NaCl), lactated Ringer's solution, a glycerol solution, a dextrose solution (e.g., 5% dextrose USP), and the like. The aqueous phase can also comprise pharmaceutically acceptable salts, such as, for example, potassium, calcium, and magnesium salts, as well as preservatives, antibiotics, anti-fungal agents, and the like, according to the intended use of the bimodal emulsion.

The aqueous phase further optionally comprises a surfactant. The surfactant reduces surface tension between the aqueous phase and the non-aqueous phase, thereby increasing the stability of the emulsion. Such a surfactant can be an anionic, cationic, nonionic, zwitterionic and/or ampholytic surfactant.

The surfactant can be, for example, egg yolk phospholipids such as lecithin (i.e., phosphatidyl choline) or Pluronics (i.e., block polymer polyols). Pluronics F-68, which has a molecular weight of about 8,000, can be employed. Ethoxylates of cholesterol, diacyl glycerol and dialkyl ether glycerol are also useful surfactants. Alkoxylated copolymers can also be prepared by alkoxylating the backbones of cholesterol, diacyl glycerol or dialkyl ether glycerol with ethylene oxide and propylene oxide. The surfactants can also be alkylphosphoryl choline or alkylglycerophosphoryl choline surfactants, such as those described in U.S. Patent No. 5,314,325, the disclosure

of which is incorporated by reference herein. Specific examples of these surfactants include 1,2-dioctylglycero-3-phosphoryl choline, 1,2-ditetradecylglycero-3-phosphoryl choline, 1,2-dihexadecylglycero-3-phosphoryl choline, 1,2-dioctadecylglycero-3-phosphoryl choline, 1-hexadecyl-2-tetradecylglycero-3-phosphoryl choline, 1-octadecyl-2-tetradecylglycero-3-phosphoryl choline, 1-tetradecyl-2-octadecylglycero-3-phosphoryl choline, 1-hexadecyl-2-octadecylglycero-3-phosphoryl choline, 1-2-dioctadecylglycero-3-phosphoryl choline, 1-octadecyl-2-hexadecylglycero-3-phosphoryl choline, 1-tetradecyl-2-hexadecylglycero-3-phosphoryl choline, 2,2-ditetradecyl-1-phosphoryl choline ethane and 1-hexadecyltetradecylglycero-3-phosphoryl choline.

Suitable anionic surfactants include alkyl or aryl sulfates, sulfonates, carboxylates or phosphates. Suitable cationic surfactants include, for example, mono-, di-, tri- and tetra-alkyl or aryl ammonium salts. Suitable non-ionic surfactants include alkyl or aryl compounds, which hydrophilic portion comprises polyoxyethylene and/or polyoxypropylene chains, sugar molecules, polyalcohol derivatives or other hydrophilic groups. Zwitterionic surfactants can be a combination of the above anionic or cationic groups, and which hydrophobic part consists of any other polymer, such as polyisobutylene or polypropylene oxides. The surfactant can also comprise a mixture of one or more of these surfactants.

In another aspect, a non-aqueous phase is provided without an aqueous phase. The non-aqueous phase comprises a mixture of the taxane, the butyrate polyalcohol ester and the Ostwald ripening stabilizer. The butyrate polyalcohol ester solubilizes the taxane in the non-aqueous mixture. The Ostwald ripening stabilizer reduces the transfer of the butyrate polyalcohol ester through the aqueous phase when the non-aqueous phase is combined with an aqueous solution to form a bimodal emulsion according to the present invention. Such a non-aqueous phase can optionally include other ingredients, such as preservatives, bioactive agents, and the like, as more fully discussed below.

In another aspect of the invention, methods of preparing bimodal emulsions are provided. Such methods generally comprise preparing the aqueous (continuous) phase, preparing the non-aqueous (disperse) phase, and/or dispersing the non-aqueous phase into the aqueous phase. The dispersed phase comprises the taxane, the butyrate polyalcohol ester the Ostwald ripening stabilizer, and optionally any other components. The non-aqueous phase can be formed by any suitable method for mixing hydrophobic components. For example, a mixture can be formed by adding the taxane to

the butyrate polyalcohol ester while mixing. The Ostwald ripening stabilizer can be added to butyrate polyalcohol ester before, during or after the taxane is added. A carrier can also be used to aid in dissolving the taxane. Suitable carriers include alcohols, such as isopropanol, or other relatively low boiling point (i.e., volatile) solvents. Following dissolution of the taxane, the carrier can be removed, for example, by rotary evaporation or by evaporation under a stream of nitrogen.

Following preparation of the aqueous phase and the non-aqueous phase, the emulsion is prepared by dispersing the non-aqueous phase in the aqueous phase. In one embodiment, the emulsion is prepared by dispersing the non-aqueous phase, comprising the taxane, butyrate polyalcohol ester, the Ostwald ripening stabilizer, and optionally any other components, into the aqueous phase. In another embodiment, the emulsion is prepared by dispersing the non-aqueous phase, comprising the taxane, butyrate polyalcohol ester and stabilizer, and optionally any other components, into the aqueous phase containing a surfactant. The non-aqueous phase can be dispersed in the aqueous phase by any suitable means for forming an emulsion. For example, the bimodal emulsion can be prepared by homogenization using a high speed homogenizer.

In yet another embodiment, the bimodal emulsion is prepared by dispersing in water containing the surfactant the components of the non-aqueous phase such that the butyrate polyalcohol ester, taxane and stabilizer are incorporated in the non-aqueous phase of the emulsion. While stirring at high speed, a mixture of the taxane and the butyrate polyalcohol ester is slowly added to the water. The Ostwald ripening stabilizer is then added to the emulsion. The resulting emulsion can then be refined by cycling through a homogenizer (e.g., a Microfluidizer model 110Y or Gaulin homogenizer). Alternatively, the emulsion can be formed by loss shear mixing followed by comminution in a homogenizer (e.g., a Microfluidizer model 110Y or an Emulsiflex-1,000). The emulsion can optionally be sterilized by passage through a sterile filter, such as, for example, a 0.45 or 0.22 micron sterile filter (Gelman Acrodisc, Ann Arbor, Michigan). Additional methods of preparing emulsions useful within the invention are disclosed in U.S. Patent No. 5,558,853, which is incorporated by reference herein.

The bimodal emulsion can also be formed by preparing a template emulsion comprising in a non-aqueous phase the butyrate polyalcohol ester and the Ostwald ripening stabilizer, and optionally one or more other components such as non-aqueous carriers and/or combining the template emulsion with the taxane. The taxane can be added without dilution, in the form of a solution with a carrier, or as an emulsion with

the taxane in the non-aqueous phase. The result of the combination is that the non-aqueous phase containing the taxane migrates to the template emulsion to form a bimodal emulsion comprising the taxane, the butyrate polyalcohol ester and the Ostwald ripening stabilizer in the non-aqueous phase. The non-aqueous phase can optionally include other components. This process can be carried out by, for example, metered in-line mixing, since the thermodynamics of the mixing process are such that the particle size tends to be a predictable value.

In any of the foregoing embodiments, the droplet or particle size of the non-aqueous phase can by controlled according to the method of preparing the bimodal emulsion. Changes in the droplet or particle size of the non-aqueous phase due to the addition of the taxane can be determined by calculation of the anticipated volume increase upon addition of the taxane to the non-aqueous phase. Desired droplet or particle sizes in the non-aqueous phase can also be achieved by the use of an appropriately sized filter. The average droplet or particle size is typically between about 50 to about 200 nm in diameter, although greater and lesser average diameters are within the scope of the invention. Average droplet or particle sizes in an emulsion can be determined using, for example, an Ostuku LPA-3000 (Otsuka Electronics), a Nicomp 370 or a Horiba CAPA-700 Particle Analyzer.

Methods of Using the Bimodal Emulsion

The bimodal emulsion according to the present invention is useful for treating hyperproliferative diseases. Methods are provided for the administration to a subject of a therapeutically effective amount of the emulsion to treat a hyperproliferative disease. The subject can be an animal, including but not limited to, cows, pigs, horses, chickens, cats, dogs, and the like, and is typically a mammal, and in a particular embodiment, human. In another specific embodiment, a non-human mammal is the subject.

The emulsion can be administered as a therapeutically effective emulsion by any suitable route known to the skilled artisan including, for example, intravenous, intrathecal, oral, mucosal, nasal, parental, anal, and the like. The emulsion can be formulated with neutral or salt forms for the aqueous phase. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, and the like, and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium,

ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The bimodal emulsion can further comprise a diluent, adjuvant, preservative, bioactive agent, an additional stabilizer, a vehicle or a viscogen. Pharmaceutical diluents can be sterile liquids, such as water when the emulsion is administered intravenously. Saline, dextrose and glycerol solutions can also be employed as diluents or vehicles, particularly for injectable solutions. Examples of suitable diluents, adjuvants, preservatives, additional stabilizers, and vehicles are described in, for example, Remington's Pharmaceutical Sciences, by E.W. Martin, which is incorporated by reference herein.

Suitable preservatives include, for example, sodium benzoate, quaternary ammonium salts, sodium azide, methyl paraben, propyl paraben, sorbic acid, ascorbylpalmitate, butylated hydroxyanisole, butylated hydroxytoluene, chlorobutanol, dehydroacetic acid, ethylenediamine, monothioglycerol, potassium benzoate, potassium metabisulfite, potassium sorbate, sodium bisulfite, sulfur dioxide, and organic mercurial salts. Suitable viscogens include, for example, carboxymethylcellulose, sorbitol, dextrose, and polyethylene glycols.

Suitable bioactive agents include, for example, antineoplastic agents, such as platinum compounds (e.g., spiroplatin, cisplatin, and carboplatin), methotrexate, adriamycin, mitomycin, ansamitocin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopolylysine, vincristine, busulfan, chlorambucil, melphalan (e.g., PAM, L-PAM or phenylalanine mustard), mercaptopurine, mitotane, procarbazine hydrochloride dactinomycin (actinomycin D), daunorubicin hydrochloride, doxorubicin hydrochloride, mitomycin, plicamycin (mithramycin), aminoglutethimide, estramustine phosphate sodium, flutamide, leuprolide acetate, megestrol acetate, tamoxifen citrate, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase), Erwina asparaginase, etoposide (VP-16), interferon α-2a, interferon α-2b, teniposide (VM-26), vinblastine sulfate (VLB), vincristine sulfate, bleomycin, bleomycin sulfate, methotrexate, adriamycin, and arabinosyl; blood products, such as parenteral iron, hemin, hematoporphyrins and their derivatives; biological response modifiers, such as muramyldipeptide, muramyltripeptide, microbial cell wall components, lymphokines (e.g., bacterial endotoxin such as lipopoly-saccharide, macrophage activation factor), sub-units of bacteria (such as Mycobacteria and Corynebacteria), and the synthetic dipeptide N-acetyl-muramyl-L-alanyl-D-isoglutamine.

Other suitable bioactive agents include anti-fungal agents, such as ketoconazole, nystatin, griseofulvin, flucytosine, miconazole, amphotericin B, ricin, and β-lactam antibiotics (e.g., sulfazecin); hormones and steroids such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone. betamethasone acetate and betamethasone sodium phosphate, vetamethasone disodium phosphate, vetamnethasone sodium phosphate, cortisone acetate, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, flunsolide, hydrocortisone, hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, paramethasone acetate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide and fludrocortisone acetate; vitamins such as cyanocobalamin neinoic acid, retinoids and derivatives such as retinol palmitate, and α-tocopherol; peptides, such as manganese super oxide dismutase; enzymes such as alkaline phosphatase; anti-allergic agents such as amelexanox; anti-coagulation agents such as phenprocoumon and heparin; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as para-aminosalicylic acid, isoniazid, capreomycin sulfate cycloserine, ethambutol hydrochloride ethionamide, pyrazinamide, rifampin, and streptomycin sulfate; antivirals such as acyclovir, amantadine azidothymidine (AZT or Zidovudine), ribavirin, amantadine, vidarabine, and vidarabine monohydrate (adenine arabinoside, ara-A); antianginals such as diltiazem, nifedipine, verapamil, erythrityl tetranitrate, isosorbide dinitrate, nitroglycerin (glyceryl trinitrate) and pentaerythritol tetranitrate; anticoagulants such as phenprocoumon, heparin; and antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V, ticarcillin rifampin and tetracycline.

Other suitable bioactive agents include drugs, such as antiinflammatories such as difunisal, ibuprofen, indomethacin, meclofenamate, mefenamic acid, naproxen, oxyphenbutazone, phenylbutazone, piroxicam, sulindac, tolmetin, aspirin and salicylates; antiprotozoans such as chloroquine, hydroxychloroquine, metronidazole, quinine and meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric; opiates such as codeine, heroin, methadone, morphine and opium; cardiac glycosides such

as deslanoside, digitoxin, digoxin, digitalin and digitalis; neuromuscular blockers such as atracurium besylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride (suxamethonium chloride), tubocurarine chloride and vecuronium bromide; sedatives (hypnotics) such as amobarbital, amobarbital sodium, aprobarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, pentobarbital sodium, phenobarbital sodium, secobarbital sodium, talbutal, temazepam and triazolam; local anesthetics such as bupivacaine hydrochloride, chloroprocaine hydrochloride, etidocaine hydrochloride, lidocaine hydrochloride, mepivacaine hydrochloride, procaine hydrochloride and tetracaine hydrochloride; and general anesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium and thiopental sodium.

To treat hyperproliferative disease, emulsions according to the present invention are administered by rapid as by injection or over a period of time as by slow infusion or administration of slow release formulations. In one embodiment, the bimodal emulsion is formulated in accordance with routine procedures for intravenous administration to human beings. Typically, emulsions for intravenous administration are solutions in sterile isotonic aqueous buffer or a dextrose solution. Where necessary, the emulsion can also include a local anesthetic to ease pain at the site of the injection. The emulsions can be administered daily and/or weekly, according to the desired treatment regimen.

In another aspect, the emulsion can be administered locally to the area in need of treatment. This administration can be achieved by, for example, local infusion during surgery, topical application (e.g., in conjunction with a wound dressing after surgery), by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes such as silastic membranes, or fibers. In another embodiment, administration can be by direct injection at the site (or former site) of, for example, a malignant tumor or neoplastic or pre-neoplastic tissue.

In yet another embodiment, the emulsion can be delivered in a controlled release system. For example, a pump can be used (see, e.g., Langer, Science 249:1527-33 (1990)); Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). Similarly, polymeric

materials can be used (see, e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, Medical Applications of Controlled Release, 2:115-38 (1984)). Other controlled release systems are discussed in, for example, the review by Langer (supra).

For treating tissues in the central nervous system, administration can be by injection or infusion into the cerebrospinal fluid (CSF). When it is intended that the emulsion be administered to cells in the central nervous system, administration can be with one or more other components capable of promoting penetration of the emulsion across the blood-brain barrier. Pulmonary administration can also be employed, such as, for example, by use of an inhaler or nebulizer, and formulation of the emulsion with an aerosolizing agent.

The emulsion can also be administered orally or rectally in an acceptable dosage form including, but not limited to, capsules, tablets, caplets, lozenges, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating aids, such as magnesium stearate, may be added. For administration in a capsule form, useful diluents include lactose and dried corn starch. If desired, certain sweeteners, flavorants or colorants can also be used.

Nasal administration is typically performed using a solution as a nasal spray and can be dispensed by a variety of methods known to those skilled in the art. Systems for intranasally dispensing liquids as a spray are well known (see e.g., U.S. Patent No. 4,511,069, which is incorporated by reference herein). Preferred nasal spray solutions comprise the emulsion in a liquid carrier that optionally include a nonionic surfactant for enhancing absorption of the drug and one or more buffers or other additives to minimize nasal irritation. In some embodiments, the nasal spray solution further comprises a propellant. The pH of the nasal spray solution is typically between about pH 6.8 and 7.2.

For intranasal administration, compositions which improve the absorption of nasally administered bimodal emulsion and reduce nasal irritation, especially when

used in a chronically administered treatment protocol, are desirable. In this context, the utilization of surface-active agents to enhance absorption of the emulsion is preferred. (See, e.g., Hirai et al., Int. J. Pharmaceutics 1:173-84 (1981); Great Britain Patent Specification 1 527 605, each of which is incorporated by reference herein.) However, nasal administration of drugs enhanced by surfactants can be accompanied by nasal irritation, including stinging, congestion and rhinorrhea. Thus, compositions which enhance absorption through the nasal mucosa with reduced irritation are desirable.

To achieve this goal, a combination of surfactants can be used. Nonionic surfactants such as nonoxynol-9, laureth-9, poloxamer-124, octoxynol-9 and lauramide DEA are particularly useful. Nonoxynol-9 (N-9) is an ethoxylated alkyl phenol, the polyethyleneoxy condensate of nonylphenol with 9 moles of ethylene oxide. This surfactant has been used in detergent products and is sold under trade names such as, SURFONIC® N-95 (Jefferson), NEUTRONYX® 600 (Onyx) and IGEPAL® (CO-630 (GAF). N-9 is considered to be a hard detergent. N-9 has also been used as a spermatocide (The Merck Index, 10th Ed., Entry 6518). To minimize irritation attributed to employment of surfactants, one or more anti-irritant additives are included in the emulsion. In one example, polysorbate-80 has been shown to reduce the irritation caused by intranasally administered drugs where delivery was enhanced by used of a nonionic surfactant (see, e.g., U.S. Patent No. 5,902,789, which is incorporated by reference herein).

The amount of the emulsion in a dosage form will vary, depending upon the nature of the taxane and the butyrate polyalcohol ester and the dosage form. The specific dosage and treatment regime for any particular subject, or disease, will depend upon a variety of factors, including the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the judgment of the treating physician and the severity of the particular disease being treated. The amount of taxane and the butyrate polyalcohol ester will also depend upon their specific activities and whether the emulsion is co-administered with any other therapeutic or prophylactic ingredients. Dosage levels of between about 0.001 and about 100 mg/kg body weight per day, typically between about 0.1 and about 10 mg/kg body weight per day are useful.

Generally, the emulsion can be supplied either as separate phases (e.g., aqueous and nonaqueous phases) or as a unitary dosage form. For example, the emulsion can be supplied in concentrated form in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent (e.g., taxane and butyrate

polyalcohol ester). Where the emulsion is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the emulsion is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

In another aspect, the invention provides a pharmaceutical pack or kit comprising one or more containers filled with the emulsion and/or carriers, diluents, vehicles and the like. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

The following examples are offered to illustrate, but no to limit the claimed invention.

Example 1:

A bimodal emulsion is formed. The non-aqueous phase comprises 20 weight percent tributyrin and 5 weight percent triolein as the Ostwald ripening stabilizer. The non-aqueous phase further comprises 10 mg/ml paclitaxel. The aqueous phase comprises normal saline solution and phosphatidyl choline as a surfactant. The emulsion is formed by dispersing the non-aqueous phase in the aqueous phase by high speed homogenization. The average droplet or particle size of the non-aqueous phase is about 50-200 nm.

Example 2:

Bimodal emulsions are formed that have different ratios of the butyrate polyalcohol ester, Ostwald ripening stabilizer, and taxane. The taxane ranges from about 0.1 to about 20 mg/ml. The butyrate polyalcohol ester ranges from about 0.5% to about 25% w/v. The Ostwald ripening stabilizer is present at about 0.005% to about 6.25% w/v. The aqueous phase is water, normal saline, D_5W, or a dextrose i.v. solution. The emulsions are formed in a homogenizer (e.g., a Microfluidizer model 110Y or an Emulsiflex-1,000). The emulsions are then sterilized by passage through a 0.45 or 0.22 micron sterile filter.

Example 3:

The short term physical stability of the emulsions of Example 2 is measured by dilution in i.v. solutions, such as D_5W, 10% dextrose and normal saline. The emulsions are diluted by factors of 10; 100 and 1000 followed by agitation. Diluted control emulsions, without the Ostwald ripening stabilizer, are also prepared. The diluted emulsions are examined for stability by visual inspection and by measuring average particle size distribution. Average particle size distribution is examined every 3 hours for 48 hours.

Example 4:

The long term physical stability of the emulsions of Example 2 is measured by incubation at room temperature and at 4°C. Control emulsions, without the Ostwald ripening stabilizer, are also prepared. The emulsions are examined for stability by visual and by measuring average particle size distribution weekly over period of 6 months.

Example 5:

To confirm the in vivo effectiveness of the bimodal emulsions, female B6D2F mice are subcutaneously injected with 10^7 B16 melanoma tumor cells. Four days after implantation, the mice are randomly sorted into groups of 10 mice per group. Each group is administered with an i.v. saline solution, an i.v. saline solution with TAXOL®, or the bimodal modal emulsions of Example 2 in an i.v. saline solution. The i.v. administration schedule is one every three days for a total of 5 dosages. The bimodal emulsions of Example 2 are administered as a bolus injection. The TAXOL® solution is infused over 2 minutes following 10-fold dilution with saline. The volume of liquids administered are about 7-8 milliliters per kilogram body weight. Anti-tumor activity is assessed according to the guidelines established by the National Cancer Institute (Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials and Approval, B.A. Teicher (ed.), Humana Press, New Jersey, pp. 101-125 (1997)).

Other suitable assays and models, as well as refinements, are disclosed in the following references: Khleif et al., J. Immunother. 22:155-65 (1999); Ressing et al., J. Immunol. 154:5934-43 (1995); Feltkamp et al., Eur. J. Immunol. 23:2242-49 (1993); Zhu et al., Scand. J. Immunol. 42:557-63 (1995); Mayordomo et al., Nat. Med. 1:1297-302 (1995); Van Driel et al., Eur. J. Cancer 35:946-52 (1999); Ji et al., Int. J. Cancer

78:41-45 (1998); Feltkamp et al., Eur. J. Immunol. 25:2638-42 (1995); Kast et al., J. Immunol. 152:3904-12 (1994); Ressing et al., Immunotechnology 2:241-51 (1996); Metlief et al., Curr. Opin. Immunol. 8:651-57 (1996); and Vierboom et al., J. Immunother. 21:399-408 (1998), the disclosures of which are incorporated by reference herein.

Example 6:

The bimodal emulsion of Example 1 is administered to a human subject having breast cancer. The emulsion is administered intravenously as a 20 milliliter bolus comprising 200 mg of paclitaxel. Progression of the cancer is followed by mammography, ultrasound and biopsy.

Although the foregoing invention has been described in detail by way of example for purposes of clarity of understanding, it will be apparent to the artisan that certain changes and modifications are comprehended by the disclosure and may be practiced without undue experimentation within the scope of the appended claims, which are presented by way of illustration not limitation.

WHAT IS CLAIMED IS:

1. A sterile, pharmaceutically acceptable taxane emulsion comprising
a continuous aqueous phase;
a surfactant; and
a dispersed non-aqueous phase, the dispersed phase comprising a taxane, a butyrate polyalcohol ester and an insoluble oil in an amount sufficient to stabilize the emulsion.

2. The taxane emulsion of claim 1, wherein the taxane is paclitaxel; taxotere; spicatin; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone, acetate; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone; taxane-2β, 5β, 9β, 10β-tetrol, cyclic 9, 10-acetal with acetone; taxane; cephalomannine-7-xyloside; 7-epi-10-deacetylcephalomannine; 10-deacetylcephalomannine; cephalomannine; taxol B; 13-(2', 3'-dihydroxy-3'-phenylpropionyl)baccatin III; yunnanxol; 7-(4-Azidobenzoyl)baccatin III; N-debenzoyltaxol A; O-acetylbaccatin IV; 7-(triethylsilyl)baccatin III; 7, 10-Di-O-[(2,2,2-trichloroethoxy)carbonyl]baccatin III; baccatin III 13-O-acetate; baccatin diacetate; baccatin; baccatin VII; baccatin VI; baccatin IV; 7-epi-baccatin III; baccatin V; baccatin I; baccatin III; baccatin A; 10-deacetyl-7-epitaxol; epitaxol; 10-deacetyltaxol C; 7-xylosyl-10-deacetyltaxol; 10-deacetyltaxol-7-xyloside; 7-epi-10-deacetyltaxol; 10-deacetyltaxol; 10-deacetyltaxol B; or a combination thereof.

3. The taxane emulsion of claim 1, wherein the continuous aqueous phase is water, a saline solution, a dextrose solution, or a glycerol solution.

4. The taxane emulsion of claim 1, wherein the surfactant is an .anionic surfactant, a cationic surfactant, a nonionic surfactant, a zwitterionic surfactant or an amphoteric surfactant.

5. The taxane emulsion of claim 1, wherein the butyrate polyalcohol ester is tributyrin, tetrabutyral butanol, glycerol dibutyrate, tetrabutyral pentanol, or pentabutyral pentanol.

6. The taxane emulsion of claim 1, further comprising a diluent, preservative, or bioactive agent.

7. The taxane emulsion of claim 1, wherein the insoluble oil comprises a medium chain length triglyceride.

8. The taxane emulsion of claim 1, wherein the non-aqueous phase comprises droplets or particles having an average diameter of 50 to 200 nm.

9. A stable taxane emulsion comprising

a continuous aqueous phase comprising a surfactant; and

a dispersed non-aqueous phase, the dispersed phase comprising a therapeutically effective amount of a taxane, and a therapeutically effective amount of a butyrate polyalcohol ester, the butyrate polyalcohol ester capable of transfer through the aqueous phase to cause Ostwald ripening of the emulsion;

wherein the dispersed phase further comprises a medium chain length triglyceride that is soluble in the dispersed phase, but insoluble in the aqueous phase, and is present in an amount effective to reduce Ostwald ripening of the emulsion.

10. The taxane emulsion of claim 9, wherein the taxane is paclitaxel; taxotere; spicatin; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone, acetate; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-,cyclic 9, 10-acetal with acetone; taxane-2β, 5β, 9β, 10β-tetrol, cyclic 9, 10-acetal with acetone; taxane; cephalomannine-7-xyloside; 7-epi-10-deacetylcephalomannine; 10-deacetylcephalomannine; cephalomannine; taxol B; 13-(2',3'-dihydroxy-3'-phenylpropionyl)baccatin III; yunnanxol; 7-(4-Azidobenzoyl)baccatin III; N-debenzoyltaxol A; O-acetylbaccatin IV; 7-(triethylsilyl)baccatin III; 7, 10-Di-O-[(2,2,2-trichloroethoxy)carbonyl]baccatin III; baccatin III 13-O-acetate; baccatin diacetate; baccatin; baccatin VII; baccatin VI; baccatin IV; 7-epi-baccatin III; baccatin V; baccatin I; baccatin III; baccatin A; 10-deacetyl-7-epitaxol; epitaxol; 10-deacetyltaxol C; 7-xylosyl-10-deacetyltaxol; 10-deacetyltaxol-7-xyloside; 7-epi-10-deacetyltaxol; 10-deacetyltaxol; 10-deacetyltaxol B; or a combination thereof.

11. The taxane emulsion of claim 9, wherein the continuous aqueous phase is water, a saline solution, a dextrose solution, or a glycerol solution.

12. The taxane emulsion of claim 9, wherein the surfactant is an anionic surfactant, a cationic surfactant, a nonionic surfactant, a zwitterionic surfactant or an amphoteric surfactant.

13. The taxane emulsion of claim 9, wherein the butyrate polyalcohol ester is tributyrin, tetrabutyral butanol, glycerol dibutyrate, tetrabutyral pentanol, or pentabutyral pentanol.

14. The taxane emulsion of claim 9, whereby the taxane inhibits proliferation of at least one cell of a subject.

15. The taxane emulsion of claim 9, whereby the butyrate polyalcohol ester induces apoptosis by at least one cell of a subject.

16. The taxane emulsion of claim 9, wherein the non-aqueous phase comprises droplets or particles having an average diameter of 50 to 200 nm.

17. A non-aqueous composition, comprising:
a taxane;
a butyrate polyalcohol ester for solubilizing the taxane; and
an Ostwald ripening stabilizer comprising a medium chain length triglyceride to reduce Ostwald ripening of the composition when present in an aqueous solution.

18. The composition of claim 17, wherein the taxane is paclitaxel; taxotere; spicatin; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone, acetate; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-,cyclic 9, 10-acetal with acetone; taxane-2β, 5β, 9β, 10β-tetrol, cyclic 9, 10-acetal with acetone; taxane; cephalomannine-7-xyloside; 7-epi-10-deacetylcephalomannine; 10-

deacetylcephalomannine; cephalomannine; taxol B; 13-(2',3'-dihydroxy-3'-phenylpropionyl)baccatin III; yunnanxol; 7-(4-Azidobenzoyl)baccatin III; N-debenzoyltaxol A; O-acetylbaccatin IV; 7-(triethylsilyl)baccatin III; 7, 10-Di-O-[(2,2,2-trichloroethoxy)carbonyl]baccatin III; baccatin III 13-O-acetate; baccatin diacetate; baccatin; baccatin VII; baccatin VI; baccatin IV; 7-epi-baccatin III; baccatin V; baccatin I; baccatin III; baccatin A; 10-deacetyl-7-epitaxol; epitaxol; 10-deacetyltaxol C; 7-xylosyl-10-deacetyltaxol; 10-deacetyltaxol-7-xyloside; 7-epi-10-deacetyltaxol; 10-deacetyltaxol; 10-deacetyltaxol B; or a combination thereof.

19. The composition of claim 17, wherein the butyrate polyalcohol ester is tributyrin, tetrabutyral butanol, glycerol dibutyrate, tetrabutyral pentanol, or pentabutyral pentanol.

20. A method for treating a hyperproliferative disease, comprising:

administering to a patient in need thereof a therapeutically effective amount of a stable bimodal emulsion, the emulsion comprising a continuous aqueous phase and a dispersed non-aqueous phase, the aqueous phase comprising a surfactant, the dispersed phase comprising a taxane and a butyrate polyalcohol ester, the butyrate polyalcohol ester capable of transfer through the aqueous phase to cause Ostwald ripening of the emulsion; wherein the dispersed phase further comprises an Ostwald ripening stabilizer that is soluble in the dispersed phase, but insoluble in the aqueous phase, and is present in an amount effective to reduce Ostwald ripening of the emulsion.

21. The method of claim 20, wherein the administration is intravenous, intrathecal, oral, mucosal, nasal, parental, or anal.

22. The method of claim 20, wherein the emulsion is administered as a bolus.

23. The method of claim 20, wherein the butyrate polyalcohol ester is tributyrin, tetrabutyral butanol, glycerol dibutyrate, tetrabutyral pentanol, or pentabutyral pentanol.

24. The method of claim 20, wherein the taxane is paclitaxel; taxotere; spicatin; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-, cyclic 9, 10-acetal with acetone, acetate; taxane-2, 13-dione, 5β, 9β, 10β-trihydroxy-,cyclic 9, 10-acetal with acetone; taxane-2β, 5β, 9β, 10β-tetrol, cyclic 9, 10-acetal with acetone; taxane; cephalomannine-7-xyloside; 7-epi-10-deacetylcephalomannine; 10-deacetylcephalomannine; cephalomannine; taxol B; 13-(2',3'-dihydroxy-3'-phenylpropionyl)baccatin III; yunnanxol; 7-(4-Azidobenzoyl)baccatin III; N-debenzoyltaxol A; O-acetylbaccatin IV; 7-(triethylsilyl)baccatin III; 7, 10-Di-O-[(2,2,2-trichloroethoxy)carbonyl]baccatin III; baccatin III 13-O-acetate; baccatin diacetate; baccatin; baccatin VII; baccatin VI; baccatin IV; 7-epi-baccatin III; baccatin V; baccatin I; baccatin III; baccatin A; 10-deacetyl-7-epitaxol; epitaxol; 10-deacetyltaxol C; 7-xylosyl-10-deacetyltaxol; 10-deacetyltaxol-7-xyloside; 7-epi-10-deacetyltaxol; 10-deacetyltaxol; 10-deacetyltaxol B; or a combination thereof.

25. The method of claim 20, further comprising administration of a bioactive agent with the emulsion.

26. A method of making a stable taxane emulsion, comprising:

preparing an aqueous phase comprising a surfactant;

preparing a non-aqueous phase comprising a taxane, a butyrate polyalcohol ester and an Ostwald ripening stabilizer; and

dispersing the non-aqueous phase in the aqueous phase.

27. The method of claim 26, wherein the non-aqueous phase is formed in the aqueous phase.

28. The method of claim 26, wherein the dispersing is by high speed homogenization.

INTERNATIONAL SEARCH REPORT

Int.....onal application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/335
US CL :514/449

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/449

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,877,205 A (ANDERSSON) 02 March 1999 (02.03.99), see the entire document.	1-28
A	US 5,504,102 A (AGHARKAR ET AL) 02 April 1996 (02.04.96), see the entire document.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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